7-2008

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MARINE MAMMAL SCIENCE, 24(3): 643–663 (July 2008)

DOI: 10.1111/j.1748-7692.2008.00213.x

Use of chemical tracers to assess diet and persistent organic pollutants in Antarctic Type C killer whales

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ABSTRACT

Measuring chemical tracers in tissues of marine predators provides insight into the prey consumed and the predator's contaminant exposure. In this study, samples from Type C killer whales (*Orcinus orca*) biopsied in Antarctica were analyzed for chemical tracers (*i.e.*, stable isotopes of carbon and nitrogen, fatty acids, and persistent organic pollutants [POPs]). Profiles of these individual tracers were very different from those of killer whale populations that have been studied in the eastern North and eastern Tropical Pacific. For example, $\delta^{13}C$ and $\delta^{15}N$ stable isotope values and most POP concentrations were significantly lower in the Antarctic population. In addition, multivariate statistical analyses of both fatty acid and POP profiles found distinctly different patterns for Antarctic Type C whales compared to those from whales in the other populations. Similar assays were conducted on four species of Antarctic marine fish considered potential prey for Type C killer whales. Results were consistent with a diet of fish for Type C whales, but other species (*e.g.*, low trophic-level marine mammals or penguins) could not be eliminated as supplemental prey.

Key words: killer whale, *Orcinus orca*, diet, prey, biopsy sampling, stable isotopes, fatty acids, persistent organic pollutants, Antarctica.

Killer whales (*Orcinus orca*) occupy the top level of many marine food webs. This species is locally common in the world's oceans, particularly at the higher latitudes, and occurs in either localized resident populations or in wider ranging groups (Forney and Wade 2006). Killer whales have been well studied in certain regions, notably the eastern North Pacific (ENP) where three different forms have been identified—the marine-mammal eating "transients," the fish-eating "residents," and the "offshores" that currently are thought to eat primarily fish (Bigg 1982, Jones 2006). These "ecotypes" are genetically distinct (Hoelzel *et al.* 1998), differing in various aspects of morphology and behavior (Ford *et al.* 2000), acoustics (Barrett-Lennard *et al.* 1996), and habitat use (Ford *et al.* 1998). It has been suggested that these differences contribute to reproductive isolation and perhaps incipient speciation among the different forms (Baird *et al.* 1992).

Observations on killer whales in the Southern Hemisphere, *e.g.*, Antarctica and New Zealand, also suggest ecotypic variation (Jehl *et al.* 1980, Mikhalev *et al.* 1981, Thomas *et al.* 1981, Berzin and Vladimirov 1983, Gill and Thiele 1997, Visser 1999). Recently, Pitman and Ensor (2003) reported on the existence in Antarctic waters of three ecologically and morphologically distinct forms of killer whales that apparently also show dietary specialization. Whales designated as "Type A" are thought to feed predominantly on Antarctic minke whales (*Balaenoptera bonaerensis*), "Type B" are believed to preferentially feed on pinnipeds, but they may also occasionally supplement their seal diet with cetaceans and penguins, and "Type C" are thought to prey mainly on marine fish. Type C killer whales have been observed carrying large Antarctic toothfish (*Dissostichus mawsoni*) in their mouths (Pitman and Ensor 2003). Although field observers have also reported that Type C whales interact with other marine mammals and penguins (Pitman and Ensor 2003, Ballard and Ainley 2005), no predation has been observed. In fact, Type C killer whales in McMurdo Sound are regularly observed ignoring Adélie Penguins (*Pygoscelis adeliae*), Weddell seals (*Leptonychotes weddellii*), and Antarctic minke whales that often occur in close proximity (RLP, personal observations).

Studying the diet and trophic position of top-level marine predators, such as killer whales, is essential in understanding their role in marine food webs. Unfortunately, diet composition derived solely from field sightings of feeding events has some important limitations because these observations: (1) tend to be relatively rare, especially for fish eaters; (2) can be limited by season and weather; (3) may represent only short-term dietary habits; and (4) can be adversely and severely affected by other known biases (Tollit *et al.* 1997, Yonezaki *et al.* 2003, Ford and Ellis 2006). As a means of providing supplemental data on the feeding habits of killer whales, chemical tracers acquired *via* their prey can be measured to help identify (or conversely eliminate) certain species as likely prey. In addition, these chemical tracers can sometimes indicate broad geographic localities where foraging most likely occurred (Hooker *et al.* 2001, Krahn *et al.* 2007).

The two most common biochemical signals used for assessing trophic position and dietary preferences of marine mammals are fatty acid signature analysis of blubber (Iverson *et al.* 2004) and stable carbon and nitrogen isotope values in the epidermis (Kelly 2000). Initial studies that used fatty acid compositions to study the diet of pinnipeds (Walton *et al.* 2000) and cetaceans (Dahl *et al.* 2000) were qualitative in nature. In contrast, more recent studies using fatty acid signature analysis (Iverson *et al.* 2004) have shown that a quantitative assessment of the relative contribution of specific prey to the diets of marine mammals is possible. However, the extent to which individual fatty acids are selectively metabolized

or biosynthesized by the predator must be quantitatively established through carefully controlled captive feeding studies. Furthermore, fatty acids of killer whales are significantly stratified throughout the blubber column (Krahn *et al.* 2004) and fatty acids from the inner layers—generally believed to be the most metabolically active—are expected to be best correlated with those of ingested prey (Koopman *et al.* 1996, Olsen and Grahl-Nielsen 2003). Free-ranging cetaceans, including killer whales, are most often sampled using biopsy darting techniques that acquire only the epidermis and outer blubber layer (Hoelzel *et al.* 1998, Barrett-Lennard 2000, Ylitalo *et al.* 2001). Consequently, any inferences about cetacean diets made from measurements of fatty acids in shallow biopsy blubber samples will be qualitative in nature.

Stable isotope ratios of nitrogen ($^{15}N/^{14}N$), and to a lesser extent carbon ($^{13}C/^{12}C$), show a stepwise enrichment with each increasing trophic level in the marine environment (DeNiro and Epstein 1978, 1981; Hobson and Welch 1992). Because these carbon and nitrogen isotope values reflect food consumed and assimilated, shifts in stable isotope values can be used to provide general information about the diet of a predator. Both carbon and nitrogen isotope values in the tissues of a predator are an approximately linear combination of the isotope values of all prey items consumed and their respective percentages of the diet within a certain period of time, modified to account for trophic enrichment. The specific time period is dependent on the tissue analyzed. For example, Tieszen *et al.* (1983) found that the half-life for $\delta^{13}C$ values in gerbil tissues varied from 6.4 d in the liver to 27.6 d in muscle. Furthermore, trophic enrichment values vary for each species. The enrichment values can be roughly estimated for entire ecosystems (Wada *et al.* 1987, Hobson *et al.* 2002), but species-specific values can only be determined by feeding experiments in controlled environments (Tieszen *et al.* 1983, Hobson *et al.* 1996) or carefully monitored natural systems (Fox-Dobbs *et al.* 2007). Thus, even for specialist predators such as killer whales, stable isotope measurements can reveal whether a diet inferred from field observations is reasonable, but cannot be used to establish the proportions of specific prey species consumed (Herman *et al.* 2005).

Patterns of persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), dichlor-diphenyl-trichloroethane (DDTs), and polybrominated diphenylethers (PBDEs), have been shown to differentiate cetacean stocks (Muir *et al.* 1996; Krahn *et al.* 1999, 2004), presumably due to differences in relative concentrations of POPs in their prey. However, only a few studies have compared patterns or levels of POPs in predators from the Antarctic marine food web to those in similar species from lower latitudes in the Southern and in the Northern Hemispheres. For example, Connell *et al.* (1999) found that pinnipeds from the Southern Hemisphere had considerably lower POP levels than similar species from the Northern Hemisphere. Additionally, Antarctic minke whales (Aono *et al.* 1997) and albatrosses (Muir *et al.* 2002) that feed in Antarctica have been shown to have lower POP concentrations than related species in the North Pacific. Weber and Goerke (1996) reported that POP concentrations (*i.e.*, p,p′-DDE, *trans*-nonachlor, PCB congeners 138, 153, and 180) in fish from Antarctica were substantially lower than those in fish from the North Sea, but that the concentration of hexachlorobenzene (HCB) was similar in fish from the two areas. In addition, Weber and Goerke (2003) examined how patterns of selected POPs in marine fish differed among species with different feeding habits and how these patterns changed over time. Furthermore, Goerke *et al.* (2004) found that Weddell seals and southern elephant seals (*Mirounga leonina*) greatly biomagnified certain POPs relative to Antarctic krill

(*Euphausia superba*) and that POP patterns present in the blubber of these pinnipeds also changed with increasing trophic level. Unfortunately, POP levels and patterns cannot be directly and quantitatively compared among killer whales and their likely prey, because individual POPs are biomagnified to a different extent and biomagnification factors appear to be species specific (Fisk *et al.* 2001, Hoekstra *et al.* 2003). To date, no biomagnification factors have been reported for killer whales. Thus, until these values are known, only qualitative comparisons are possible.

In the first study of this kind for Antarctic killer whales, chemical tracers (*i.e.*, stable isotopes of carbon and nitrogen, fatty acids, and POPs) were measured in biopsy samples taken from free-ranging Type C killer whales in the southwestern Ross Sea. To put these results into a more global perspective, they were compared to those from previous studies of killer whales from the ENP and eastern Tropical Pacific Ocean (ETP). In addition, samples from four Antarctic marine fish species were also analyzed for the same chemical tracers and the results were used to infer whether these species were part of the Type C killer whale diet.

METHODS

Killer Whales Sampled

Biopsies of Type C killer whales ($n = 28$) were collected from the ice edge in McMurdo Sound, off the west side of Ross Island in the southern Ross Sea, Antarctica, during the austral summer months in two consecutive years, 2005 and 2006 (Table 1). All samples were obtained from live whales using remote biopsy sampling techniques (Hoelzel *et al.* 1998, Barrett-Lennard 2000, Ylitalo *et al.* 2001) and biopsy tips of various lengths (typically 3.0–3.5 cm). All biopsy samples were stored frozen at −80°C until analyzed. In an attempt to standardize sample size, frozen biopsy samples were subjected to two lateral cuts. First, the epidermis was removed by cutting the sample 1–2 mm from the inside edge of the epidermis and then a second lateral cut was made 2 cm from the inside edge of the epidermis (sample length ~1.8 cm). The blubber and epidermis biopsy samples from the whales listed in Table 1 were analyzed for fatty acids, POPs, and stable isotopes of carbon and nitrogen. To date, no stranded killer whales have been examined in Antarctica in order to assess the blubber thickness of this population. However, in the ENP, numerous strandings of killer whales have occurred and blubber thicknesses on the upper back behind the dorsal fin was measured to be in the range of 5–8 cm for a typical adult whale. Because Type C whales are now known to be a substantially smaller form of killer whale (Pitman *et al.* 2007), it is unlikely that the blubber thickness of the Antarctic population is comparable to that of killer whales in the ENP. However, a 2-cm biopsy sample is still unlikely to include a substantial quantity of the metabolically active inner-blubber layer.

Blubber samples from Antarctic Type C killer whales of both sexes were analyzed for POPs. However, only the POP results for the adult male killer whales ($n = 7$) were compared to other populations of adult male killer whales. Reproductive females transfer a portion of their contaminant burden to their calves, so POP concentrations in females are generally lower than in males and are dependent on the number of times they have given birth (Ross *et al.* 2000, Ylitalo *et al.* 2001, Borga *et al.* 2004).

Table 1. Collection date, age class, and location for Antarctic Type C and eastern Tropical Pacific killer whale biopsy samples analyzed for fatty acids (FA), stable isotopes (SI), and persistent organic pollutants (POPs).

Sample	FA	SI	POPs[a]	Collection date	Age class	SWFSC ID[b]
Antarctic Type C[c]						
Males						
1	x	x	x	1/24/2005	Adult	Z45799
2	x	x	x	1/24/2005	Adult	Z45800
3	x	x	x	1/24/2005	Adult	Z45803
4	x	x	x	1/24/2005	Adult	Z45804
5	x	x	x	1/25/2005	Adult	Z45805
6	x	x	x	1/25/2005	Adult	Z45806
7	x	x		1/31/2006	Subadult	Z53852
8	x	x		1/31/2006	Subadult	Z53854
9	x	x		2/1/2006	Adult	Z53851
10	x	x		2/1/2006	Subadult	Z53859
11	x	x	x	2/2/2006	Adult	Z53857
12	x	x		2/2/2006	Subadult	Z53860
13	x	x		2/2/2006	Subadult	Z53861
Females						
14	x	x		1/21/2005	Adult	Z45808
15	x	x		1/21/2005	Adult	Z45809
16	x	x		1/21/2005	Adult	Z45810
17	x	x		1/21/2005	Adult	Z45811
18	x	x		1/21/2005	Adult	Z45812
19	x	x		1/21/2005	Adult	Z45813
20	x	x		1/24/2005	Adult	Z45801
21	x	x		1/24/2005	Adult	Z45802
22	x	x		1/25/2005	Adult	Z45807
23	x	x		1/23/2006	Unknown	Z53862
24	x	x		1/23/2006	Unknown	Z53863
25	x	x		1/23/2006	Unknown	Z53864
26	x	x		2/1/2006	Unknown	Z53855
27	x	x		2/1/2006	Unknown	Z53856
28	x	x		2/1/2006	Unknown	Z53858
Eastern Tropical Pacific (used for comparison)[c]						
Males						
1			x	9/26/2003	Adult	Z38168
2			x	9/26/2003	Unknown	Z38170
3			x	9/26/2003	Unknown	Z38175

[a]Although POP analyses were conducted for all killer whales in this table, only adult males "x" were used for POP concentrations (Table 4) and PCB profiles (Fig. 3). One Adult male (Z53851) was not included in POP analyses due to low percent lipid (<5%).

[b]Southwest Fisheries Science Center specimen identification number for Antarctic Type C and Eastern Tropical Pacific samples.

[c]Positions for samples collected: Antarctica, 21 January 2005, (77°32′S, 165°45′E); 24 and 25 January 2005, (77°32′S, 165°16′E); 23 January 2006, (77°33′S, 165°58′E); 31 January 2006, (77°33′S, 165°56′E); 1 February 2006, (77°27′S, 165°36′E); 2 February 2006, (77°29′S, 165°57′E and 77°27′S), 165°45′E. Eastern Tropical Pacific, 26 September 2003, (10°58′N, 88°40′W).

Killer Whale Prey Sampled

Samples of one Antarctic minke whale and four species of marine fish that were potential prey of the Antarctic Type C killer whales were also collected from McMurdo Sound, Ross Sea, Antarctica, and were analyzed for fatty acids, POPs, and stable isotopes. Fish species included: dusky notothen (*Trematomus newnesi, n* = 2 adults), bald notothen (*Pagothenia borchgrevinki*, *n* = 2 adults), Antarctic toothfish (*n* = 1 adult), and Antarctic silverfish (*Pleuragramma antarcticum*, *n* = 5 juveniles). Specimens were collected in January 2005, except the silverfish collected in 2000 and the minke whale biopsied in 2006. Unfortunately, tissue samples from other potential prey species (marine fish and mammals) were not available at the time of this study.

Fatty Acid, Stable Isotope, and POP Analyses

Blubber samples from the killer whales and minke whale were analyzed for fatty acids and POPs and the epidermis was analyzed for stable isotopes. Individual, whole fish were ground to a homogenous mixture and subsamples of the homogenate were analyzed for fatty acids, POPs, and stable isotopes.

Fatty acid concentrations were determined as reported by Krahn *et al*. (2004) and fatty acid concentration data were expressed as weight percent of total fatty acids. The n-number standard nomenclature system was used for abbreviating the names of these fatty acids, where the number following the "n" symbol in the abbreviation refers to the carbon position of the first double bond relative to the alkyl end of the molecule. A full list of all 83 fatty acids measured as part of this study, as well as their abbreviations, systematic, and trivial names can be found in Table 1 of Krahn *et al.* (2004). These 83 fatty acids, listed by n-number nomenclature, are also found in Appendix S1.

Stable isotope analyses were conducted as described previously by Herman *et al.* (2005). All tissues were lipid extracted prior to stable isotope analysis. Stable isotope values were expressed in δ notation as parts per mil (‰) by the following expression:

$$\delta Z = [(Rsample/Rstd) - 1] \times 1,000$$

where Z represents ^{15}N or ^{13}C and *Rsample* is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$ for the tissue sample. Here, *Rstd* is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$ of the reference standard. All nitrogen values were referenced to atmospheric nitrogen ($\delta^{15}N$ for atmospheric N_2 is 0‰ exactly) and carbon values were referenced to Vienna Pee Dee Belemnite (a.k.a. $\delta^{13}C$ of NBS 19 ≡ 1.95‰, Coplen *et al.* 2006). The daily laboratory standards were calibrated using the following primary standard values: NBS-22 ($\delta^{13}C = -30.03$), IAEA-CH-6 ($\delta^{13}C = -10.45$), IAEA-N-1 ($\delta^{15}N = 0.43$), and IAEA-N-2 ($\delta^{15}N = 20.39$). Maximum standard deviations allowed for replicate analyses for $\delta^{13}C$ and $\delta^{15}N$ in the calibration standards were ≤±0.3‰ and ≤±0.2‰, respectively.

POP concentrations were determined using the procedure of Sloan *et al*. (2004). A total of 40 PCB congeners, 24 chlorinated pesticides, and 10 PBDE congeners were determined in these samples; see Sloan *et al.* (2004) for a list of all POP analytes measured. In this article, ∑PCBs is the sum of all 40 PCB congeners analyzed; ∑DDTs is the sum of o,p′,p′-DDD, p,p′-DDE, o,p′-DDE, o,p′-DDT, and p,p′-DDT; ∑chlordanes is the sum of oxychlordane, *gamma*-chlordane, nonachlor III, *alpha*-chlordane, *trans*-nonachlor, and *cis*-nonachlor; ∑hexachlorocyclohexanes

($\sum$HCHs) is the sum of *alpha*-, *beta*-, and *gamma*-HCH isomers; and finally, $\sum$PBDEs is the sum of congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 183. Total lipids, as well as lipid classes, were measured in the samples by a TLC-FID method (Ylitalo *et al.* 2005).

Statistical Analyses

Unless indicated otherwise, all multivariate and univariate analyses were conducted on nontransformed data using either JMP Statistical Discovery Software (PC profession edition, version 5.01) or Primer-e (PC edition, version 6.1.6). Differences in absolute POP levels were examined by comparing POP concentrations expressed on a lipid-normalized basis (ng/g total lipid). In addition, individual PCB congener concentration data were expressed as weight percent of all 40 PCBs to normalize the results so that any differences in measured PCB patterns was independent of absolute tissue concentrations. All principal component analyses (PCA) were performed on the correlation matrix computed from these compositional data.

Multidimensional scaling analysis (MDS) of the fatty acid data was performed using the Primer-E statistical software package. Prior to MDS analysis, the fatty acid weight percent composition data were pretreated by: (1) normalizing the weight percent of each individual fatty acid among all samples within the data set, (2) computing an among-sample dissimilarity matrix based on Euclidean distances between the variables, and (3) subjecting the dissimilarity matrix to classical multidimensional scaling analysis. The sample-to-sample proximity values (dissimilarities) resulting from the MDS analysis were then plotted in the form of a two-dimensional perceptual map.

RESULTS

Stable Isotope Values

The mean δ^{13}C and δ^{15}N values for Type C killer whales (Table 2) were significantly lower ($P < 0.05$) compared to Northern Hemisphere killer whale groups. Approximate trophic levels were calculated with Equation 4 from Hodum and Hobson (2000) using δ^{15}N values for three different fish-eating killer whale populations and regionally specific zooplankton data from the literature (Schell *et al.* 1998, Kline 1999, Hodum and Hobson 2000). The results were: Antarctic Type C killer whales = 5.5; eastern Aleutian Island residents = 5.3; Gulf of Alaska residents = 5.1.

Four species of possible Antarctic fish prey had measured δ^{13}C values that were similar to each other (Table 3; Fig. 1) and, among all the killer whale groups listed, were most similar to those measured in the Type C killer whale population. The Antarctic silverfish, dusky notothen, and bald notothen also had mean δ^{15}N values that were very similar to each other (Table 3; Fig. 1). These prey were about one trophic level lower than Type C whales, assuming δ^{15}N enrichment values of about +3.3 units per trophic level in this ecosystem (Wada *et al.* 1987) and δ^{13}C enrichment of +1.3 units per trophic level (Hobson *et al.* 1996, 2002). The δ^{15}N and δ^{13}C values for the "Antarctic Type C estimated diet" were calculated by subtracting the δ^{15}N and δ^{13}C values trophic enrichment values from those for Type C whales (Fig. 1). The single Antarctic toothfish had a higher δ^{15}N value than that found for all but one of the Type C whales (Table 2, 3).

Table 2. Mean (±1 SD) carbon and nitrogen stable isotope values in epidermis of selected killer whale populations.[a]

SWFSC ID	Sex	$\delta^{13}C$	$\delta^{15}N$
Antarctic Type C males[b]			
Z45799	Adult	−23.7 ± 0.1	13.3 ± 0.3
Z45800	Adult	−24.1 ± 0.1	13.1 ± 0.2
Z45803	Adult	−23.6 ± 0.2	13.4 ± 0.3
Z45804	Adult	−23.8 ± 0.1	14.1 ± 0.2
Z45805	Adult	−23.9 ± 0.1	12.8 ± 0.2
Z45806	Adult	−23.5 ± 0.1	13.7 ± 0.2
Z53852	Subadult	−24.1 ± 0.1	13.2 ± 0.3
Z53854	Subadult	−24.1 ± 0.1	13.4 ± 0.3
Z53851	Adult	−23.9 ± 0.1	13.1 ± 0.3
Z53859	Subadult	−24.0 ± 0.1	12.9 ± 0.3
Z53857	Adult	−24.0 ± 0.1	13.3 ± 0.3
Z53860	Subadult	−24.0 ± 0.1	12.9 ± 0.3
Z53861	Subadult	−23.7 ± 0.1	12.9 ± 0.3
Mean for males ($n = 13$)		−23.9 ± 0.2	13.2 ± 0.4
Antarctic Type C females[b]			
Z45808	Adult	−23.8 ± 0.1	13.5 ± 0.2
Z45809	Adult	−23.9 ± 0.1	13.3 ± 0.2
Z45810	Adult	−23.6 ± 0.2	13.6 ± 0.2
Z45811	Adult	−23.7 ± 0.1	13.7 ± 0.2
Z45812	Adult	−24.0 ± 0.1	12.6 ± 0.2
Z45813	Adult	−23.8 ± 0.1	13.0 ± 0.2
Z45801	Adult	−23.8 ± 0.1	12.9 ± 0.2
Z45802	Adult	−23.5 ± 0.2	13.6 ± 0.3
Z45807	Adult	−23.4 ± 0.1	13.5 ± 0.2
Z53862	Unknown	−23.8 ± 0.1	13.4 ± 0.3
Z53863	Unknown	−24.1 ± 0.1	12.8 ± 0.3
Z53864	Unknown	−24.2 ± 0.1	12.8 ± 0.3
Z53855	Unknown	−23.9 ± 0.1	12.5 ± 0.3
Z53856	Unknown	−23.9 ± 0.1	13.0 ± 0.3
Z53858	Unknown	−23.9 ± 0.1	13.1 ± 0.3
Mean for females ($n = 15$)		−23.8 ± 0.2	13.2 ± 0.4

Comparison groups	Sample size	$\delta^{13}C$	$\delta^{15}N$
Alaska residents	79	−16.7 ± 1.3	16.4 ± 1.5
Alaska offshores	5	−16.8 ± 0.3	17.0 ± 0.3
Alaska transients	47	−16.3 ± 0.8	17.7 ± 1.5
West Coast (California) transients	10	−15.9 ± 0.6	18.7 ± 1.2

[a]Values for Alaska residents, offshores and transients, and West Coast transients are from Herman *et al*. (2005) and Krahn *et al.* (2007).

[b]Both carbon and nitrogen values were statistically significantly different ($P < 0.05$) from those of the other killer whale populations.

Table 3. Mean (±1 SD) stable isotope values measured in possible Antarctic prey species, as well as literature values for additional potential prey species.

Animal ID (n)	Tissue	$\delta^{13}C$	$\delta^{15}N$
Antarctic fish (measured values)			
Dusky notothen ($n = 2$; small adults)	Whole	−25.2, −24.7	10.4, 11.4
Bald notothen ($n = 2$; small adults)	Whole	−26.0, −24.3	10.1, 11.2
Antarctic silverfish ($n = 5$; juveniles)	Whole	−24.3 ± 0.3	10.3 ± 0.4
Antarctic toothfish ($n = 1$; adult)	Muscle	−26.3	14.0
Antarctic minke whale ($n = 1$)	Skin	−24.3	7.6
Antarctic prey (literature values)			
Antarctic silverfish *(Pleuragramma antarcticum; n* = 13; adults)[a]	Whole	−23.9 ± 0.7	10.7 ± 1.2
Deepwater notothen *(Trematomus loennbergii; n* = 2)[b]	Muscle	−26.8, −24.5	10.3, 13.4
Weddell seals *(Leptonychotes weddellii; n* = 12)[b]	Plasma	−25.5 ± 0.1	13.1 ± 0.2
Eelpout *(Rhigophilia dearborni; n* = 1)[b]	Muscle	−23.6	13.2
Emerald rockcod *(T. bernacchii; n* = ?)[c]	?	−23.4	10.4
Adélie penguin (*Pygoscelis adeliae;* Prydz Bay*; n* = 8)[d]	Muscle	−23.4 ± 0.1	9.3 ± 0.2
Icefish (*Chionodraco hamatus; n* = 8)[d]	?	−22.5 ± 0.3	12.6 ± 0.3
Crabeater seal (*Lobodon carcinophagus; n* = 30; adult)[e]	Serum	−26.5 ± 1.0	8.4 ± 0.6
Ross seal (*Ommotophoca rossii; n* = 21)[e]	Serum	−24.3 ± 0.4	10.6 ± 0.6
Leopard seal (*Hydrurga leptonyx; n* = 2)[e]	Serum	−24.8 ± 0.3	12.3 ± 0.5
Antarctic krill *(Euphausia superba; n* = 12; adults)[f]	Muscle	−29.8 ± 0.6	3.6 ± 0.2
Adélie penguin (*Pygoscelis adeliae;* Palmer Station; *n* = 6)[f]	Muscle	−23.7 ± 0.3	12.5 ± 1.6

[a]Hodum and Hobson (2000), [b]Burns *et al.* (1998), [c]Wada *et al.* (1987), [d]Hall-Aspland *et al.* (2005), [e]Zhao *et al.* (2004), [f]Dunton (2001), [g]Cherel and Hobson (2007).

Fatty Acid Compositions and Profiles

Among the 83 individual fatty acids measured in the blubber of the Antarctic killer whales, only 55 were routinely present at weight percent values above the method quantitation limits (*ca.*, 0.01%; see Appendix S1). Consequently, in this study, a fatty acid profile is defined as the multidimensional vector comprising the fatty acid weight percent values for each of these 55 fatty acids and includes both exogenous (dietary) and endogenous (nondietary) fatty acids. Mean summed weight percent values are also reported in Appendix S1 for several classes of fatty acids measured in individual Antarctic Type C killer whales and their potential prey.

Similarities in the fatty acid profiles among Antarctic Type C and three other killer whale populations (offshore, Alaska resident, and Alaska transient; Herman *et al.* 2005, Krahn *et al.* 2007) were evaluated by subjecting these compositional data to a multidimensional scaling analysis and depicting the similarities among the groups as a two-dimensional perceptual map (Fig. 2).

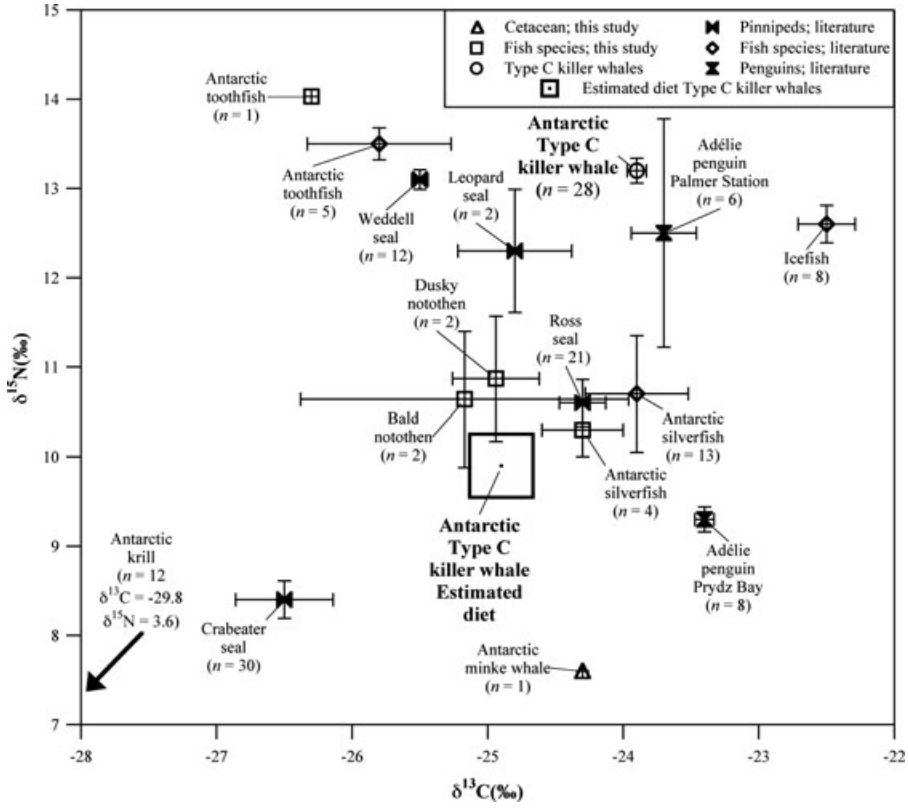

Figure 1. Nitrogen and carbon stable isotope values for Type C killer whales and potential Antarctic prey species (mean ± 95% confidence intervals). See Table 1 for references for those carbon and nitrogen stable isotope values obtained from the literature.

POP Concentrations and Patterns

Mean concentrations in all groups of POPs (Table 4, Appendix S2) were significantly higher ($P < 0.05$) in adult male Type C killer whales than in Type C females. Because POP concentrations in killer whales generally increase with age in males (Ross *et al.* 2000), their concentrations are often highly variable. POP concentrations (ng/g lipid) in adult male Type C killer whales were also compared to those in adult males for other groups of killer whales—offshores, Alaska residents, and Alaska transients, West Coast (California) transients and ETP whales (Table 4). Type C whales had the lowest mean concentrations for all groups of POPs, except for HCB. For example, the mean $\sum$PCB and $\sum$DDT concentrations in adult male Type C whales, relative to those from the other five killer whale groups (Table 4), ranged from 0.23% to 12% and from 0.12% to 20%, respectively. Conversely, mean HCB concentrations measured in Type C whales were comparable to those in ENP residents and offshores. The Antarctic fish generally had very low concentrations of POPs (Table 4, Appendix S2), with the exception that one of the two bald notothen samples had a relatively high $\sum$PCBs level, although the other contaminant classes measured in

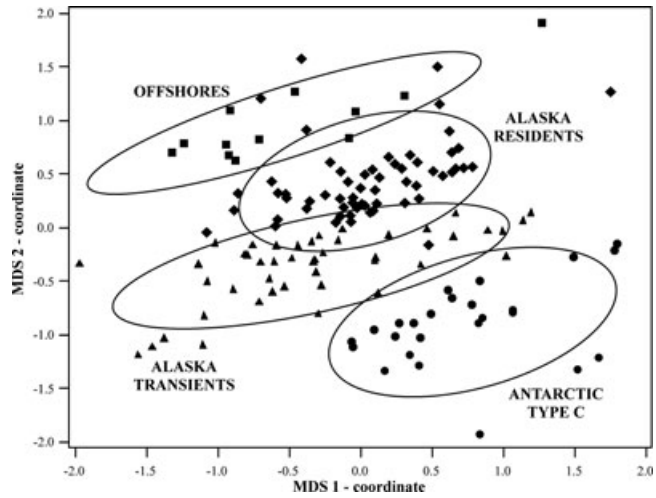

Figure 2. Two-dimensional perceptual map obtained by multidimensional scaling analysis of the fatty acid profiles of Antarctic Type C and Alaskan resident, offshore, and transient killer whales. Each point in this plot represents the fatty acid profile of an individual whale where the Euclidean distance between any two points is proportional to the intersample difference between their normalized fatty acid profiles. The fatty acid profiles include both exogenous (dietary) and endogenous (nondietary) fatty acids for the 55 fatty acids present above method quantitation limits. Fatty acid data for the Alaska killer whale populations were obtained from Herman *et al*. (2005) and Krahn *et al*. (2007).

this single fish sample were quite low. In addition, the Antarctic toothfish had higher concentrations of $\sum$DDTs, $\sum$chlordanes, and HCB than were found for the other Antarctic fish. Concentrations of POPs were much lower in the single minke whale than those found for the Type C whales and approximately the same magnitude as those found for the Antarctic toothfish (Table 4).

PCB weight percent composition data, *i.e.*, the PCB congener profiles, for Type C killer whales were compared to the other populations (adult males only) using a principal component analysis. A two-dimensional PCA plot was created to assess the extent to which these contaminant patterns differ among these spatially and ecologically distinct groups (Fig. 3). In this plot, the first and second principal components (PC1 and PC2) accounted for 54% and 20% of the total variance, respectively. The relative orientations of the eigenvector projections corresponding to the 12 PCB congeners responsible for the observed separations among individuals and among groups are also depicted (Fig. 3). The PCB profiles of Antarctic Type C killer whales were highly dissimilar to those of the other four killer whale populations, largely because the former had a high relative abundance of higher chlorinated PCB congeners in their blubber tissues, particularly congeners PCB 149 and PCB 170.

Discussion

Antarctic Type C killer whales were shown to have fatty acid, stable isotope, and POP profiles that were very different from those of the ENP and ETP killer whale populations. Although Type C whales have only been observed to feed on

Table 4. Persistent organic pollutant concentrations and ranges (ng/g lipid) and percent lipid in blubber from Antarctic Type C killer whales and a minke whale, and in whole bodies of possible fish prey species, as well as in blubber of other populations of killer whales.

	% lipid	ΣPCBs	ΣDDTs[a]	ΣChlordanes[a]	ΣHCHs[a]	HCB	ΣPBDEs[a]
Type C killer whales							
Males[b] ($n = 7$)							
Mean ± SD	16.9 ± 7.7	1,600 ± 1,100	4,300 ± 2,900	1,300 ± 780	<LOQ	740 ± 330	12 ± 28
Range	(8.4–31.7)	(540–3,600)	(1,700–10,000)	(600–2,900)	<LOQ	(440–1,300)	(<LOQ–74)
Male subadults ($n = 5$)							
Mean ± SD	12.4 ± 1.0	2,100 ± 2,300	3,300 ± 3,100	1,200 ± 1,100	<LOQ	670 ± 400	4.2 ± 9.4
Range	(10.9–13.4)	(310–5,300)	(160–7,200)	(30–2,400)	<LOQ	(42–1,100)	(<LOQ–21)
Females ($n = 15$)							
Mean ± SD	17.4 ± 5.8	600 ± 430	1,200 ± 610	530 ± 280	<LOQ	440 ± 260	4.1 ± 11
Range	(8.6–27.5)	(93–1,500)	(170–2,500)	(57–1,100)	<LOQ	(50–1,000)	(<LOQ–39)
Antarctic fish							
Dusky notothen ($n = 2$)							
Range	(8.5/1.1)	(25/160)	(5.9/39)	(1.4/12)	<LOQ	(18/39)	<LOQ
Bald notothen ($n = 2$)							
Range	(4.1/2.4)	(52/3,400)	(26/130)	(7.2/54)	<LOQ	(22/58)	<LOQ
Antarctic silverfish ($n = 4$)[c]							
Mean ± SD	4.5 ± 3.3	42 ± 30	<LOQ	<LOQ	<LOQ	20 ± 6	<LOQ
Range	(1.8–9.3)	(5.5–71)	<LOQ	<LOQ	<LOQ	(11–25)	<LOQ
Antarctic toothfish ($n = 1$)	27.8	20	41	29	<LOQ	46	<LOQ
Antarctic minke whale ($n = 1$)	16.2	130	56	14	<LOQ	80	<LOQ

Continued

Table 4. Continued

	% lipid	ΣPCBs	ΣDDTs	ΣChlordanes	ΣHCHs	HCB	ΣPBDEs
Other killer whale populations (for comparisons)[d]							
Alaska residents ($n = 40$)	25.4 ± 10.8	13,000 ± 5,900	21,000 ± 12,000	6,200 ± 2,400	520 ± 200	640 ± 270	76 ± 70
Offshores ($n = 4$)	17.9 ± 3.7	110,000 ± 22,000	420,000 ± 100,000	16,000 ± 2,300	500 ± 87	570 ± 130	3,300 ± 940
Alaska transients ($n = 15$)	21.3 ± 7.7	120,000 ± 49,000	200,000 ± 110,000	71,000 ± 24,000	9,800 ± 3,400	3,600 ± 2,600	790 ± 590
West Coast (California) transients ($n = 4$)	13.8 ± 4.4	630,000 ± 190,000	3,700,000 ± 910,000	56,000 ± 12,000	4,800 ± 1,100	1,600 ± 620	12,600[e]
Eastern Tropical Pacific ($n = 3$)	7.4 ± 4.5	20,000 ± 8,300	1,200,000 ± 500,000	4,100 ± 1,600	120 ± 41	160 ± 53	36[e]

[a]For mean and standard deviation calculations, <LOQ (limit of quantitation) was set = 0.
[b]Sample Z53851 was not included in POPs analyses due to low percent lipid (<5%).
[c]One of the silverfish had insufficient sample for POP analysis.
[d]Results for adult males only; from Krahn *et al.* (2007).
[e]$n = 1$.

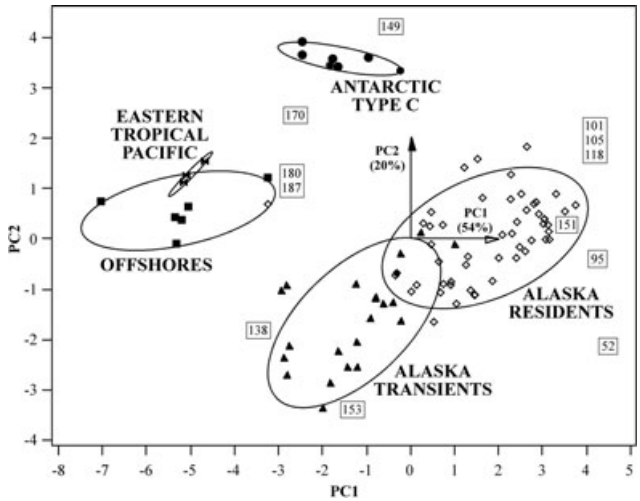

Figure 3. Principal component (PC) analysis depicting the differences in the PCB profiles of adult male killer whales from the Antarctic, eastern North Pacific, and eastern Tropical Pacific zones. The relative orientations of the eigenvectors corresponding to the 12 specific PCB congeners responsible for the observed separation among the regional killer whale groups and ecotypes are also shown. PCB data for the Alaska killer whale populations were obtained from Herman *et al*. (2005) and Krahn *et al*. (2007).

marine fish, chemical tracer results from this study suggested that other prey groups (*e.g.*, lower trophic-level marine mammal, penguin, or cephalopod species) could be important constituents of their overall diet. Ostensibly, the significantly lower values for nitrogen stable isotopes would seem to indicate that Type C whales feed at a trophic level that is lower than those of the fish-eating killer whale populations studied in the other regions (Herman *et al.* 2005, Krahn *et al.* 2007). However, when the approximate trophic levels for the presumed fish-eating Type C whales were calculated using established correction factors (Schell *et al.* 1998, Kline 1999, Hodum and Hobson 2000), Type C and ENP resident killer whales occupied approximately the same trophic level (5.5 and 5.3, respectively). Thus, the lower-than-expected carbon and nitrogen stable isotope values observed in the Antarctic Type C killer whales (Table 2, Fig. 1) were likely the result of the much reduced $\delta^{13}C$ and $\delta^{15}N$ values that exist at the base of the food chain in the coastal Antarctic ecosystem (Goericke and Fry 1994, Cabana and Rasmussen 1996). With respect to Antarctic $\delta^{13}C$ values in particular, the low values can be partly explained by the decrease in ^{13}C enrichment that generally occurs with decreasing water temperatures (Sackett *et al.* 1965).

The stable isotope values offer some insight into the possible prey species of Type C killer whales. The $\delta^{15}N$ values in the single adult Antarctic toothfish collected for this study ($\delta^{15}N = 14.0$, $n = 1$), as well as in toothfish reported by Burns *et al.* (1998) ($\delta^{15}N = 13.5 \pm 0.2$, $n = 5$) were very similar to those for Type C killer whales. Thus, the two species appear to feed at nearly the same trophic level, decreasing the likelihood that adult toothfish are a major diet component of Type C whales. This apparent exclusion of Antarctic toothfish as a primary prey species was rather unexpected because, even though there have been very few observations

of feeding to date, toothfish have been the only prey observed (Pitman and Ensor 2003, Ainley *et al.* 2006). On the other hand, stable isotope analyses indicated that three other common Antarctic nototheniids (Antarctic silverfish, dusky notothen, and bald notothen) had carbon and nitrogen isotope values that were about one trophic level lower than values measured in Type C whales. Therefore, those species (individually or in combination) may be major components in the diet of Type C whales, in particular, the silverfish that represent more than 90% of the abundance and biomass of the midwater fish fauna in the waters of the Ross Sea (DeWitt 1970, La Mesa *et al.* 2004). Silverfish and the two notothens are all small species (maximum length 28 cm) that killer whales would probably swallow whole underwater, whereas the toothfish are large enough (up to at least 175 cm and 100 kg; Collins 2006) that large individuals are likely to be brought to the surface to be eaten or shared (Ford and Ellis 2006). Thus, when interpreting Type C prey preferences, the very visible consumption of toothfish might account for the seeming contradiction between the field observations and stable isotope results.

Predation on pinnipeds, other cetaceans, or penguins—or a mixed diet including both fish and nonfish species—could also be consistent with these stable isotope data. Unfortunately, tissue samples from other potential prey species were not available for this study. A very recent study (Lauriano *et al.* 2007) conducted in the eastern Ross Sea of Antarctica reported observations of killer whale feeding behavior they interpreted as being consistent with predation on high biomass, schooling fish (*e.g.*, notothenoids). Thus, although the prey species analyzed for this study are by no means inclusive of all the potential prey of Type C killer whales, they represent many of the species currently believed to be likely prey of these whales.

The fatty acid profiles of Type C killer whales as a group were substantially different from those of Alaska killer whales (Fig. 2). Although some overlap was evident among individuals from each of the four populations, previous studies (Herman *et al.* 2005, Krahn *et al.* 2007) have demonstrated that a simple linear combination of five specific individual fatty acids was sufficient to unambiguously classify (separate) each of the three Alaska killer whale ecotypes from one another using discriminant function analysis. Somewhat surprisingly, the profiles indicated that Type C killer whales were marginally more similar to the mammal-eating transients than they were to the fish-eating populations in Alaska. These results could indicate that the Antarctic Type C killer whales supplement a fish diet with marine mammal prey. Alternatively, the observation may result from (1) highly dissimilar fatty acid compositions in the fish species that inhabit these two largely dissimilar ecosystems; or (2) differential stratification of individual fatty acids within the blubber column owing to factors such as highly dissimilar oceanographic conditions (*e.g.*, temperature, salinity). Interestingly, Type C whales have not been observed preying on other marine mammals and penguins (Pitman and Ensor 2003, Ballard and Ainley 2005), even when these species occur in close proximity (RLP, personal observation). Thus, additional samples of other potential prey species (including nonfish prey) must be analyzed and fatty acid stratification in the blubber of Type C killer whales must be fully characterized to enable the fatty acid profiles to be more informatively compared.

With the exception of the highly volatile pesticide HCB, mean lipid-normalized POP concentrations in adult male Antarctic Type C killer whales (Table 4) were much lower than those reported for the fish-eating Alaska resident or offshore killer whales (Herman *et al.* 2005, Krahn *et al.* 2007). These results were not surprising considering the isolation of Antarctica and its small human population (ranges from about 1,000 people in the austral winter to a high of 4,000 in the austral summer).

Presumably, a majority of these pollutants have been transported to the region *via* the atmosphere or oceanic currents (Wania and Mackay 1996). Although it is not currently known if Type C whales are resident in Antarctica or whether they migrate to lower latitudes during the winter (Pitman and Ensor 2003), migration could increase their exposure to pollutants. Regardless, Type C killer whales have the lowest levels of POPs (except HCB) of any killer whale population studied to date. In contrast, when POP concentrations in adult male Type C killer whales were compared to those of male Antarctic minke whales sampled in western Antarctica (1992/1993, $n = 20$; Aono *et al.* 1997) and to the single Antarctic minke whale biopsied in 2006 (Table 4), $\sum$PCBs, $\sum$DDTs, $\sum$chlordanes, and HCB were found to be several times higher in the killer whales. Most of the differences in concentrations in the two species were likely due to the low levels of contaminants in krill that dominate the minke whale diet compared to higher levels of contaminants in fish or other higher trophic level species that comprise the Type C diet. Although modest increases in pollution levels were documented in both western and eastern Antarctica between the mid-1980s and early 1990s (Aono *et al.* 1997, Goerke *et al.* 2004), the minke whale blubber collected in 2006 (Table 4) had POP concentrations similar to or lower than those in minke whales sampled in the early 1990s. Thus, temporal trends alone are not likely to account for the large magnitude differences (~5–90-fold) observed in POP concentrations between killer and minke whales.

PCB patterns in the blubber of the Antarctic Type C killer whales were also very different from those of the other killer whale populations (Fig. 3), primarily due to a high relative abundance of higher chlorinated congeners (particularly PCB 149 and PCB 170) in Type C whales. Because point sources of pollution in Antarctica are relatively rare, the PCBs acquired by the biota from the region are generally the lower molecular weight congeners that can be transported *via* the atmosphere or ocean (Wania and Mackay 1996). However, certain areas in McMurdo Sound have been documented to contain high, but patchy concentrations of the anthropogenically introduced Aroclor 1260 (one of several technical mixtures of PCBs) that contains the higher chlorinated congeners (Risebrough *et al.* 1990, Geochemical and Environmental Research Group 2003). This may explain why blubber of the adult male Type C killer whales contained high molecular weight PCB congeners characteristic of Aroclor 1260, as well as smaller proportions of the lower molecular weight congeners typical of atmospheric transport.

In summary, chemical tracer results were consistent with a diet of fish for Antarctic Type C killer whales in the southwestern Ross Sea, although these whales may supplement their diet with other species (*e.g.*, cetaceans, pinnipeds, penguins, or cephalopods). A mixed diet of fish and other marine species cannot not be ruled out until data from a sufficient number of representative samples of these prey species have been investigated. Furthermore, Type C whales had the lowest concentrations of POPs (except HCBs) yet recorded for any killer whale population, presumably due to their isolation in Antarctic waters. Additional studies that compare chemical tracers in all three killer whale ecotypes in Antarctica will be important in specifying the role of these large and abundant predators in the Antarctic marine food web.

Acknowledgments

We greatly appreciate the technical assistance of D. W. Brown, G. M. Ylitalo, C. A. Sloan, R. H. Boyer, D. Boyd, K. Tilbury, G. Yanagida, and J. L. Bolton in sample and data

analysis. N. Black of the Monterey Bay Whale Watch generously provided samples of the West Coast/California killer whales. We would also like to thank the laboratory staff of the SWFSC Population Identity Program, especially, K. Coultrup, N. M. Hedrick, A. R. Lang, and K. M. Robertson for generating a portion of the genetic data used in this manuscript. P. Cziko and A. DeVries generously provided Antarctic fish samples for analysis. Field work for RLP at McMurdo was supported by grants from National Science Foundation (OPP-0338428) and the U.S. Marine Mammal Commission. Finally, we are grateful for the funding support of T. K. Rowles from the Marine Mammal Health and Stranding Response Program of NOAA Fisheries. Killer whale biopsy samples were collected under MMPA permit 774-1714-00 and Antarctic Conservation Area permit ACA 2004-009M#2; analyses were conducted under MMPA permit 932-1489-07.

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Received: 12 February 2007
Accepted: 26 February 2008

SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article online:

Appendix S1. Fatty acid compositions[a,b] (weight percent) of individual Antarctic Type C killer whales and selected Antarctic putative prey analyzed as part of this study.

Appendix S2. Persistent organic pollutant (POP) concentrations[a,b] for Antarctic Type C killer whale biopsy and possible prey samples.